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Two major series of investigations are being undertaken to explore the metabolic responses of women who meet military standards for body-weight and percent body-fat to the nutritional stressors of food restriction. A comparison of their responses is being made with those occurring in men, who also meet the standards established by the military. The first series (Series 1) has been devoted to the temporal (progressive) changes in lipid, carbohydrate and protein metabolism that occur during a short-term (3 day) fasting period. Data obtained so far for leucine kinetics, nitrogen output, fatty acid kinetics do not reveal any significant differences, either qualitatively or quantitatively in the metabolic response to an acute total food lack in women versus men. This series of studies will be completed pending further analysis of samples obtained. Work is now underway on the second set of studies. The first phase of this 3-week hypercaloric intake/metabolic study involves the participation of both women and men who receive a hypocaloric diet for 21 days to achieve a 900 Kcal daily dietary deficit that is based on an equal restriction of both lipid and carbohydrate but with keeping protein content at a relatively generous intake of 1.2 g/kg/day. Body composition, metabolic and physical and mental performance changes, are being monitored to asses whether there are gender differences to partial and more prolonged energy restriction.

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1. Introduction

Weight loss, an index of malnutrition, occurred in trainees of the US Ranger Training Brigade. This weight loss amounted to about 15.6% of initial body weight (1) or somewhat less (12.6%) when a nutrition intervention, that included a 470 Kcal/day supplement, was provided together with additional protein (2). While this nutritional supplement apparently reduced the severity of the weight loss, the latter was highly variable among the subjects; for some it was 15-18% body weight. According to Kinney (3) the caloric equivalent of the weight loss amounted to about 970 Kcal/day in the Ranger II study and it was also concluded that; "Ranger training did not change the pattern of tissue loss seen in studies of partial starvation without such heavy physical exertion." This observation is important since it means that studies in volunteer subjects who are not enrolled in the military could serve as a useful model in order to help clarify the metabolic and physiological processes that are responsible for these changes in body weight and composition (with fat accounting for 61% of the total weight loss). Furthermore, the functional and behavioral significance of these weight changes are still poorly understood, although there was a 23% reduction in lifting strength, impaired mental performance, reduced immune function and increased infection rates indicating increased susceptibility (2).

A further issue of considerable strategic importance to the Department of Defense relates to the increasing participation of women in all aspects of military service. According to King et al (4) there are now over 1/4 million female members on active duty in the US military services, yet adequate information is lacking on the nutritional status of women serving in the Armed Forces. Furthermore, King et al (4) conclude that while the nutritional problems of military women are similar to their physically active civilian counterparts involved in sports, the nutritional problems of military women may be exacerbated by the need to meet military body-weight and percent body-fat standards. Therefore, it is critical that new knowledge be forthcoming on the metabolic and nutritional characteristics of military women in order to develop sound and effective nutrition policies and programs designed to maintain health and achieve performance goals of this growing segment of the US military.

There are sufficient data to suggest it would be highly desirable to conduct, within the context of the nutritional/metabolic/ clinical changes observed in the previous male, Ranger Studies (1,2), a detailed series of investigations on the metabolic responses, and capacity to adapt, to conditions of a dietary energy deficit in women and to compare these with those that occur in their male counterparts.

Current knowledge concerning the metabolic response of **women of normal body composition** to periods of nutritional stress, as created by short-term starvation or prolonged hypocaloric intakes, is limited. Most of the available quantitative metabolic data are based on studies in obese men and women or in non-obese men. There are reasons to anticipate that women respond differently than men under these nutritional-stress conditions. Since negative body energy balance may well develop in military women under various operational situations and this metabolic state can compromise the health and performance of military personnel, it is now critical that relevant data be generated that can serve as a basis for establishing a sound and effective **"food doctrine"** (5) for assuring the health and maintaining the performance of military women.

2. Body of Text

The hypothesis that we are exploring are as follows:

(i) Hypotheses to be Tested

1. The metabolic response to a 3-day fast and to a 3-week period of hypocaloric intake differs between healthy, military-eligible women and men. This gender-dependent response leads to a more favorable maintenance of lean body mass in the female and, in consequence, there is a lowered risk of untoward effects on health and physiologic function in women than in men.

2. The body composition of the weight loss and its underlying metabolic basis, due to either short-term fasting or prolonged-hypocaloric feeding, can be modified by the nutrient composition of the diet. In a short-term fast, a generous antecedent intake of dietary protein will spare loss of lean tissue in women and a hypocaloric diet containing relatively high proportions of protein- and lipid-derived calories will promote maintenance of body protein homeostasis and physical performance in both women and men.

The data generated from the above, hypothesis-based, studies will provide an essential scientific data base for purposes of improving the design and implementation of programs aimed at the nutritional support and maintenance of the nutritional status of women in the military.

(ii) Technical Objectives

This investigation has the following major technical objectives:

(i) To compare and contrast the progressive (temporal) alterations in lipid, glucose and protein metabolism during short-term fasting in young women and men, who meet the military body-weight and percent body-fat standards.

(ii) To test the hypothesis that a prior high intake of protein (2.5 $g.kg^{-1}.day^{-1}$) modifies the metabolic response to a short-term (3-day) fast, with preferential sparing of body protein in both women and men.

(iii) To compare and contrast (a) changes in body composition, (b) kinetics of major energy-yielding substrates and protein metabolism, under resting conditions and during and following moderate exercise, during a three-week period of hypocaloric feeding. An energy deficit of about 900 Kcals/day will be achieved for this purpose. Kinetic and metabolic studies will include feasible and novel 24-hour stable isotope tracer-indirect calorimetry protocols.

(iv) To explore the parameters outlined in (iii) above under conditions of a "balanced" reduction in energy intake in comparison with a high protein/energy (lipid) dense hypocaloric diet in women and men.

(iii) Methods Being Used

(a) Subjects

The subjects being recruited for this study are young adult men and women between the ages of 18-30yr. Subjects will be excluded from participating for the following reasons: (a) Subjects with any acute or chronic disease or who are using drugs that the physician and principal investigator decide would interfere with the normal adaptation to the proposed intervention. (b) Subjects with any physical disability that might place them at risk during the dietary modifications and tracer experiments. (c) In order to be chosen for entry into the study both the men and women will have to meet military body-weight and percent body-fat standards (5). However, because of the limited pool available at that Unit and the competing demands on them by other ongoing protocols we expect that a majority of our subjects will be drawn from the entire ethnic and racial spectrum available to us at MIT and within the Boston/Cambridge area. Recruitment procedures for those in the local community include advertisements in different living group areas at MIT. A serious attempt will be made to include the mixture of minorities and racial backgrounds that are characteristic of the local student population. The minority representation within the MIT student population is 13% (with Asian accounting for 51%, Black 39% and Hispanic 10%).

Subjects receive medical and nursing supervision throughout the entire study. The subjects are fully informed of the purpose, nature and design of the experiments and the potential hazards involved and they sign consent forms. They are allowed to continue with their normal everyday activities and requested to maintain a **relatively** constant level of physical activity during the entire experimental period.

(b) Short-term, Complete Fasting Studies

In this study subjects are fed for 5-6 days a standardized meat-free diet, providing about 45 kcal/kg/d, and 1.2 g/kg/d protein. The latter is provided via an egg-protein-based drink. Non-protein calories will be divided as 40% fat and 60% carbohydrate. The diet is "meat-free" in order

to allow a reliable additional assessment of protein breakdown from skeletal muscle, by measuring the urinary excretion of 3-methyl-histidine, just prior to, and at the end of the 3 day fast. In addition, the diet is ^{13}C-neutral, to allow a relatively steady background (natural) ^{13}C-abundance in breath CO_2, in preparation for the tracer phase of the experiment, as described previously. Subjects ingest 3 meals per day, at 0800 h, 1200 h, and 1800 h, under supervision of investigators and/or dietary staff at the M.I.T. Clinical Research Center.

The fasting/metabolic studies are conducted at the CRC and M.I.T. Medical Department (under medical supervision). Between each of the different 3-hourly isotope intravenous infusions, sedentary activity is allowed. Water is allowed ad-libitum (but must exceed 2 liters), and multi-vitamin-mineral tablets are given on a daily basis, as well as salt tablets (NaCl) (about 4 g per day) and potassium supplements (K-LYTE®) (about 40 meq per day). Vital signs are measured every 6h and gown weight will be recorded. Blood glucose and electrolytes are monitored daily.

The tracer studies involve giving constant intravenous infusions of; (a) [$^{2}H_5$]glycerol: 6.6 μmol/kg/h, (b) [6,6,$^{2}H_2$]glucose: 13.2 μmol/kg/h, (c) [1-^{13}C]leucine: 2.8 μmol/kg/h, (d) [2,2-$^{2}H_2$]palmitate: 2.4 μmol/kg/h (following binding to albumin).

Prior to each 3-hour infusion, baseline samples (blood and breath) are taken to assess background isotopic abundance in plasma molecules (glycerol, glucose, leucine, palmitate) and breath $^{13}CO_2$. Throughout each 3-hour infusion, breath samples and blood samples (8 ml) are taken for subsequent analyses. Indirect calorimetry, using a ventilated hood, will be performed during the 2nd and 3rd hour of each infusion, to assess total CO_2 production (VCO_2), energy expenditure, and the utilization of fuels.

Plasma glycerol flux is used as a marker of whole-body lipolysis and glucose flux a measure of glucose production and uptake. The "leucine" technique allows measurement of leucine oxidation, protein oxidation, protein synthesis and protein breakdown. Palmitate flux is used as a marker of free fatty acid release and reesterification. Details of methods of analysis are provided in the original cooperative agreement proposal land will not be repeated here.

(c) Status of the Short-Term Fasting Studies

All subjects have tolerated the 3d-starvation except one female volunteer who dropped from the study at the end of day 1. One female and one male volunteer received two 5h ^{13}C-Na bicarbonate infusion on each starvation day, our purpose being to obtain bicarbonate recovery data to correct oxidation data. In addition, one female and one male did not receive any tracer, but underwent the 3 starvation period in the CRC. Here breath samples were obtained as a sham to correct for substrate oxidation data.

Body composition, total CO_2 production and O_2 consumption, $^{13}CO_2$ enrichment and total urinary nitrogen data have been measured but not yet summarized in all subjects. Plasma ^{13}C-KIC (leucine metabolite), $N_{15,15}$ urea, glycerol and palmitate kinetics have been measured in four subjects and the results are described briefly below. Further, we are currently finishing the analyses of a further six subjects (3 men and 3 women).

In brief, our findings, as summarized in attached table 1 and Figs. 1-3, indicate that there were no significant differences in lipid and carbohydrate oxidation between male and female; leucine oxidation was lower in female than in male after 36-h of starvation but after that no difference was observed. Leucine turnover, an index of protein breakdown, tended to be higher in males than in female, with the largest difference between 36 and 48 hours. Due to the relatively high variability the difference was not statistically significant. Total nitrogen excretion and urea turnover rate was higher in males than in females. We are now processing additional data on glucose, free fatty acids and glycerol.

(d) Longer-term Partial Caloric Restriction Studies

This study lasts for 41 days. It consists of 3 phases. Phase 1, weight-maintenance diet, lasts 10 days; on days 9-10 a 24h-tracer study is carried on. Phase 2, 70% of previous caloric intake, follows and lasts 3 weeks; on days 17-18 and 29-30 two more 24h-tracer studies are carried

on. Phase 3, ad libitum feeding, follows and lasts 10 days. Each 24h-tracer study involves intravenous infusion of the following stable isotope tracers: 1-^{13}C-leucine, $^{2}H_5$-glycerol, $^{2}H_2$-palmitate, $^{2}H_2$-glucose and $^{15}N_2$-urea. Subjects perform 90 min of exercise cycling at 50-60% of their VO2 max (assessed previously). Blood and breath samples are obtained at 30 min intervals throughout the infusion land at 10 min intervals during the 90 min exercise and the 90 min recovery. Total CO_2 production and O_2 consumption are measured every 30 min by indirect calorimetry. Urine collection for total nitrogen measurement, are obtained at intervals. Body composition is measured by DEXA and anthropometry in phases 1 and 2. Physical performance is tested in phase 1 and at the end of phase 2. Mental performance is tested twice/week throughout the whole 41-day study. Energy balance is measured by doubly labeled water administered on day 1 and on day 21.

Seven subjects have been enrolled, 4 males and 3 females. One girl withdrew from the study on day 9, another one on day 3. Five of them completed the study. They find the study very interesting as they are all involved in sport competition.

Body composition, total CO_2 production and O_2 consumption were measured in all subjects. $^{13}CO_2$ enrichment in breath samples is being measured soon after each infusion. We started measuring tracer enrichment in plasma. We hope to complete this study by the end of 1998.

In the next two months we plan to complete sufficient plasma enrichment measurements and at that time a body of data will have been accumulated to permit a summary of the comparative metabolic responses of men and women to this reduced caloric intake.

3. Conclusions and Plans for Current Year

This project involves two major investigations, with the second of these being a complex and difficult study of the effects of a more prolonged but less severe restriction in energy intake. We are now in the human phase of this component of our studies, which involves a far more exacting and complex experimental design and will continue to study and recruit the subjects necessary to reach our scientific goals. Pilot studies of the entire system, including the dietary control and behavioral studies, have been completed and the definitive study is well underway. The short-term study is almost complete, with the observation that men and women show quite similar metabolic responses to a complete 3-day fast.

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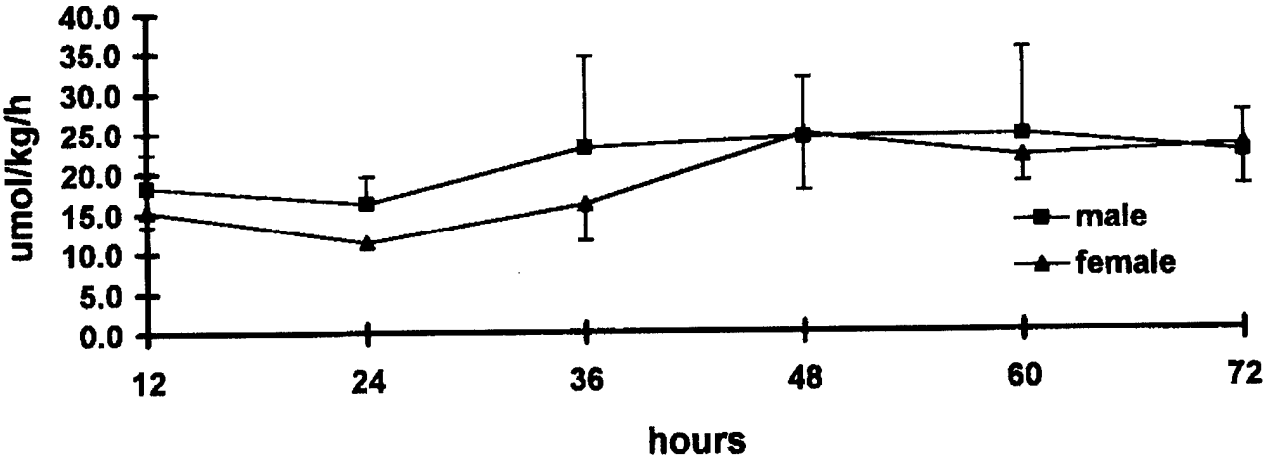
TABLE 1

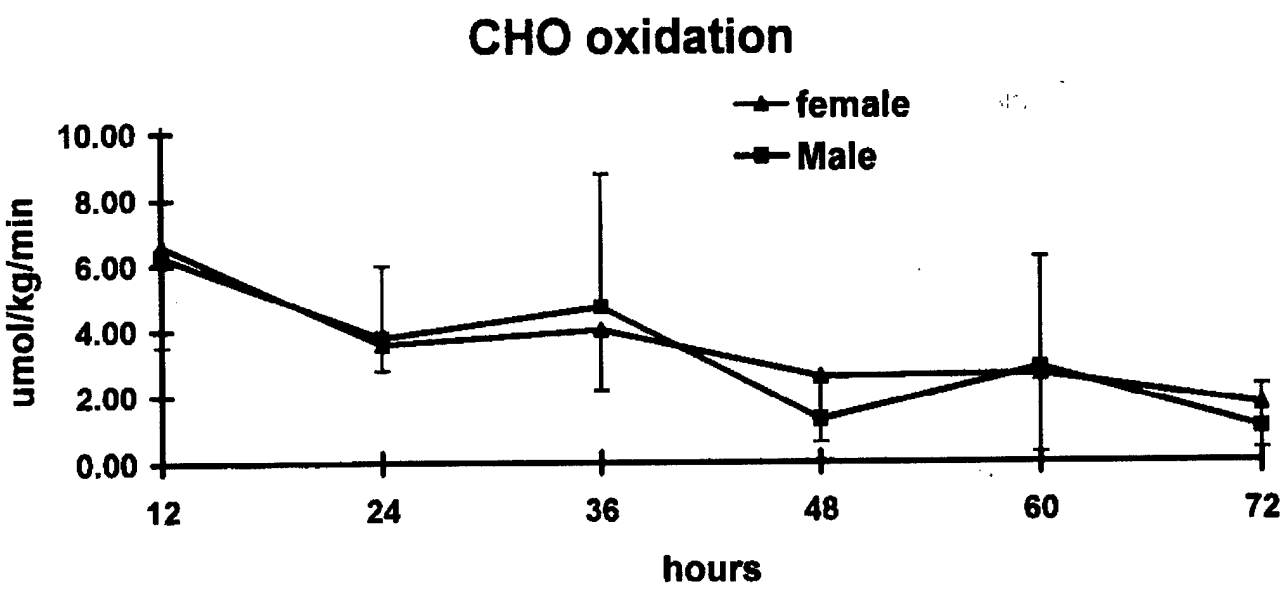
Protocol # 375: Summary of preliminary results.

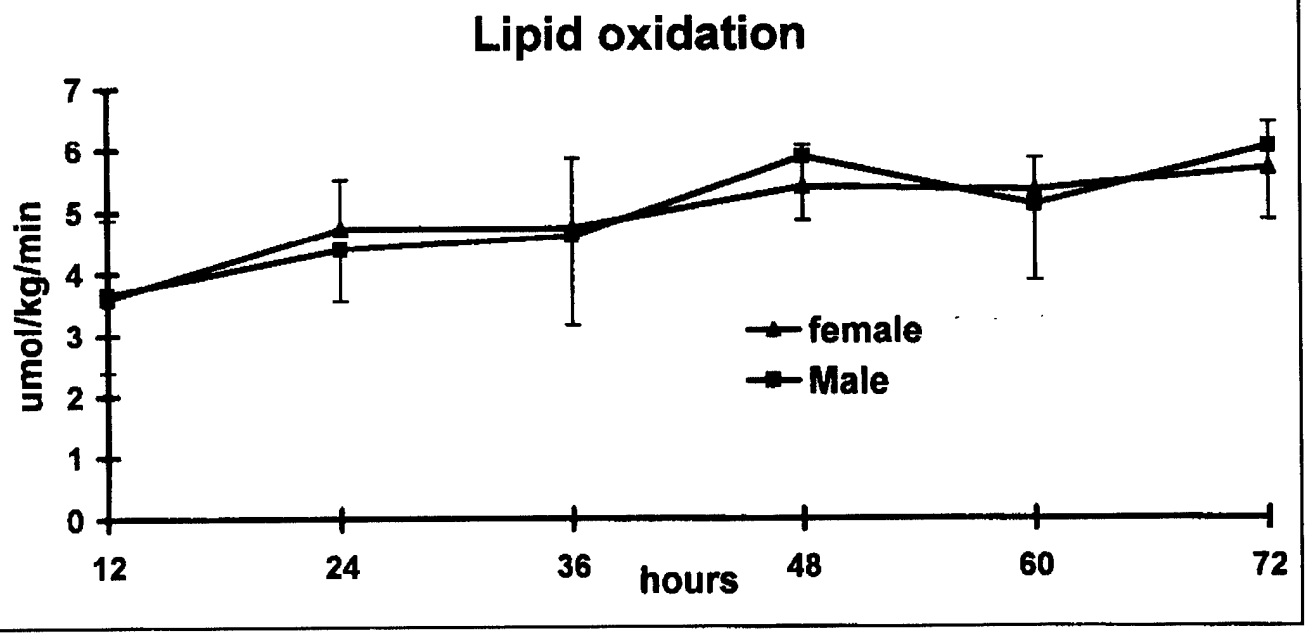
		Hours of starvation					
		12h	24h	36h	48h	60h	72h
Leucine oxidation	Male	18.3±4.1	16.1±3.4	23.0±11.4	24.2±7.4	24.3±10.8	22.0±5.0
umol/kg/h	Female	15.3±1.9	11.2±0.6	16.0±4.6	24.6±7.0	21.7±3.2	22.9±5
CHO oxidation	Male	6.3±4.0	3.8±2.2	4.7±4.0	1.2±1.3	2.8±3.4	1.0±1.3
umol/kg/min	Female	6.6±3.1	3.6±0.8	4.0±1.8	2.5±2.0	2.6±2.4	1.7±1.3
Lipid oxidation	Male	3.7±1.3	4.4±0.8	4.6±1.5	5.9±1.0	5.1±1.2	6.0±1.2
umol/kg/min	Female	3.6±1.3	4.7±0.8	4.7±1.1	5.4±0.7	5.3±0.5	5.7±0.7
Leucine Ra	Male	104.0±16.3	105.2±11.4	118.5±17.5	132.7±27.4	120.6±8.1	132.2±18.0
umol/kg/h	Female	99.5±17.2	104.4±11.1	111.5±8.9	121.2±9.5	117.4±7.2	129.4±18.1
Glucose Ra	Male	13.6±2.4	10.8±0.6	9.8±0.5	9.4±0.7	9.8±0.9	9.0±0.5
umol/kg/min	Female	14.7±1.2	12.6±0.9	10.5±0.4	9.4±0.6	9.9±0.6	9.3±0.6
Urinary nitrogen	Male	7.0±1.9	6.9±2.1	6.0±1.4	7.5±1.3	6.8±1.9	8.0±2.1
gr/d	Female	5.0±1.2	4.6±1.7	4.3±0.7	7.1±3.2	4.9±1.0	7.2±2.7
Urea Ra	Male	372.4±38.9	287.7±22.4	394.4±25.3	608.8±11.8	367.1±47.8	472.8±35.5
umol/kg/h	Female	259.6±26.8	221.1±36.4	308.9±47.8	476.0±69.1	275.8±23.0	363.3±11.2

Data are means±SD.

Fig. 1

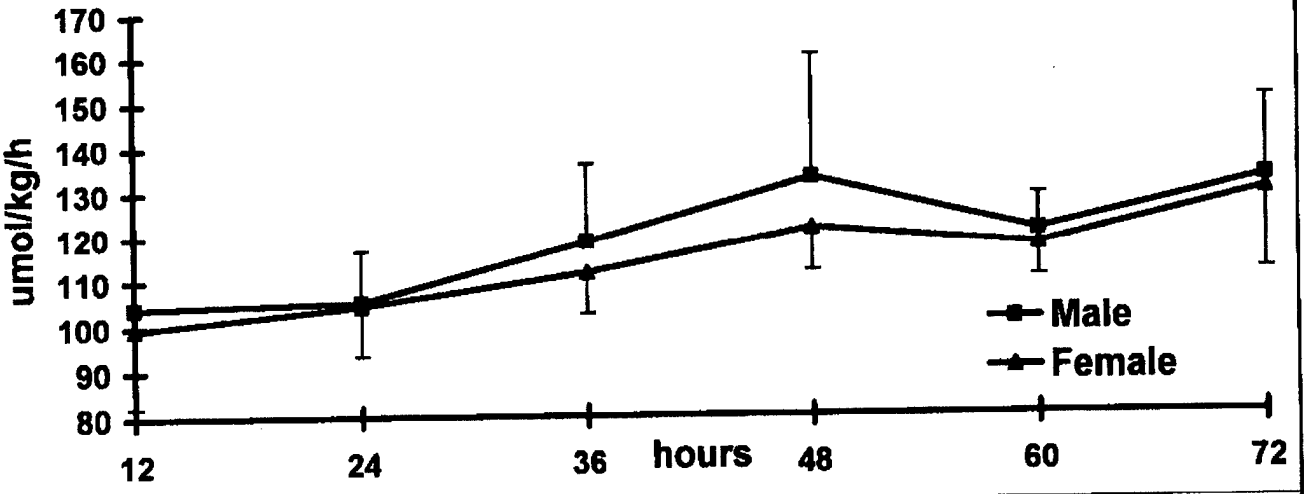

Leucine oxidation
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
umol/kg/h
male
female
12
24
36
48
60
72
hours


CHO oxidation
female
Male
10.00
8.00
6.00
4.00
2.00
0.00
umol/kg/min
12
24
36
48
60
72
hours

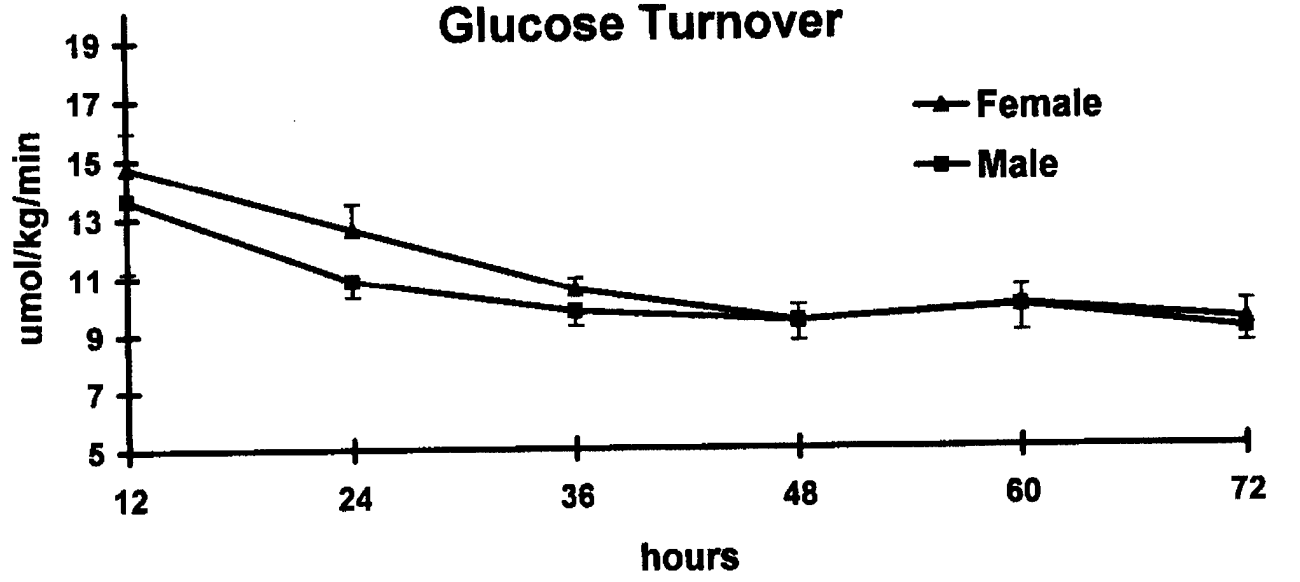

Lipid oxidation
7
6
5
4
3
2
1
0
umol/kg/min
female
Male
12
24
36
hours
48
60
72

Leucine Turnover

Fig. 2



Glucose Turnover



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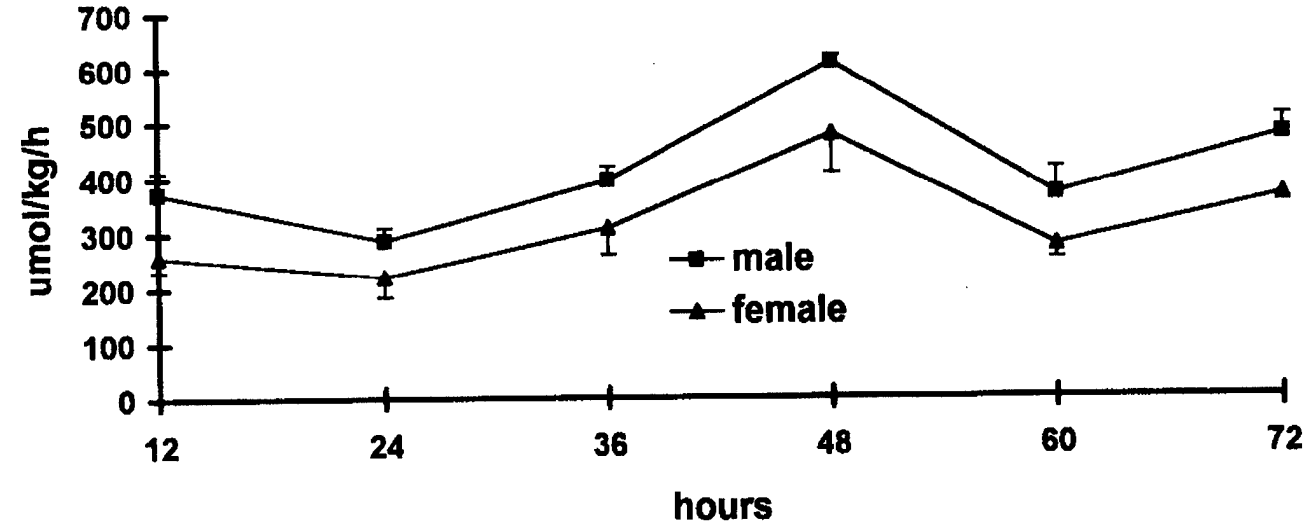

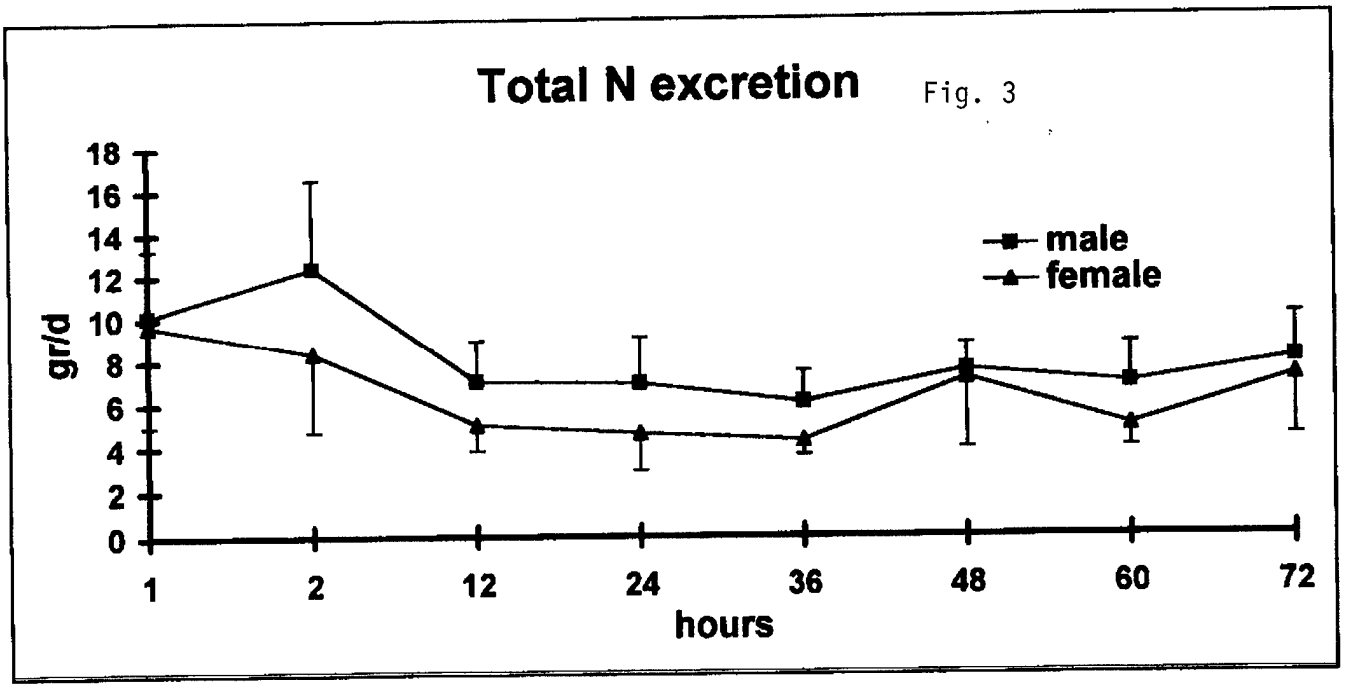
Total N excretion
Fig. 3
gr/d
18
16
14
12
10
8
6
4
2
0
male
female
1
2
12
24
36
48
60
72
hours